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STUDIES ON THE PHYSICAL PROPERTIES OF LEAVES AND LEAF SAPS.*†

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The ability of the leaves of certain species of plants to endure without injury temperatures below freezing for protracted periods of time is known to bear a close relation to the physico-chemical properties of the leaf cells. Recent advances in our knowledge of drought resistance seem to show that this property also may have its basis in the physiology of the leaf cells, rather than in anatomical peculiarities of the leaves. Studies of the physical properties of the cell and of the protoplasm may be expected to advance our knowledge of these phases of plant physiology. The relative drought resistance of different species of plants is an important factor in ecological distribution. The survival of the leaves of certain species of herbs, shrubs, and trees during the winter months can only be explained when the basis for frost resistance in the leaves of these species is understood. From the practical standpoint, knowledge of those physical properties which are correlated with frost and drought resistance will permit predictions regarding the suitability of various varieties of agricultural species for cultivation in localities subject to climatic extremes. If no physiological measures of relative frost or drought resistance be available, the alternative method of field tests must be used, comparatively costly in both time and money.

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The fundamental importance of the water relations of plants in determining their distribution has long been recognized. The universally accepted ecological classification of plants by Warming (53) into hydrophytes, mesophytes, and xerophytes recognizes this basis. Transeau (52), and later Livingston and Shreve (38, p. 518) have shown that the rainfall-evaporation ratio ("moisture ratio") is dominant in determining the distribution of vegetation in the United States. The physiological problem as to why certain plants are enabled to withstand the severe conditions of drought, while others will survive only when the water supply is moderate, and still others only when it is abundant, has by no means been completely solved.

The general presumption has been that the ability of the plant to conserve water in the leaves through the internal conditions operative in those leaves is the important factor in drought resistance. Livingston (37) and Bakke (4) believed that the "foliar transpiring power" was a fairly accurate index of the ability of leaves to conserve water, and hence of the water relations of the plant. "Transpiring power" is a measure of the rate of water vapor loss from leaves as controlled by internal conditions. Bakke concluded from his experimental work that this method "———offers an apparently adequate and somewhat simple means for classifying plant forms in a scale of xerophytism or of mesophytism." Pool (47), however, in an extended investigation, found only indifferent correlation between habitat xerophytism and "transpiring power." The importance of foliar "transpiring power" as an index of relative xerophytism has apparently been overestimated by some of the pioneers in this field.

Variations in the resistance offered by leaves of different species to water vapor loss have been explained in the past principally on the basis of the anatomical features of the leaves, such as number, size, and distribution of stomata; epidermal hairs, cuticle, compactness of tissues, schlerenchyma, etc. Pool also attempted to correlate leaf structure with "transpiring power" but his work seems to show that leaf anatomy is an inadequate explanation of variations in the resistance of leaves of different species to transpirational water loss. Some structures commonly considered characteristic of xerophytic leaves may have a negligible effect upon transpiration. Sayre (49) has shown that the abundant epidermal hairs of the mullein (*Verbascum Thapsus*), for example, have no appreciable effect

in decreasing transpiration. Some plants with leaves apparently xeromorphic, such as *Ilex opaca* (holly), *Rhododendron maximum* (rhododendron), and *Cyrtomium falcatum* (holly fern), are in habitat mesophytic. On the other hand some plants will endure extreme drought whose leaves are lacking in any xeromorphic features. Such apparent anomalies may be largely cleared up by studies of the physical properties of leaves and leaf saps, and as microchemical and microphysical methods are developed, of the protoplasm.

Maximow (41) believed that the ability of the protoplasm of the leaf cells to endure the wilted condition is the essential factor in drought resistance. By the wilted condition is meant a semi-permanent diminution in the water content of the leaf cells. This is not necessarily a visible phenomenon, since in leaves with abundant ligneous tissue wilting may be obscured. According to this worker many xerophytes are known to have high transpiration rates when the water supply is favorable, while some mesophytes have low rates of transpiration. Transpiration is, therefore, not an adequate measure of relative xerophytism. The physico-chemical properties of the protoplasm and of the cell sap must be such that the protoplasm can endure or resist this deprivation of water sufficiently to prevent coagulation of the protoplasmic colloids. The problem of drought resistance, considered from this standpoint, resolves itself into a question of why the protoplasm in the leaf cells of certain species can endure the partial desiccation involved in wilting, while the protoplasm of other species cannot.

It is now generally agreed that during the formation of ice in plant tissues, water usually freezes first in the films on the outer surface of the cell walls, bounding the intercellular spaces. As the freezing process proceeds, water gradually moves from the interior of the cell into the cell walls, replacing that which was withdrawn from the walls by the process of crystallization. It is this loss of water from the cell which is the cause, directly or indirectly, of injuries accompanying the formation of ice crystals in the tissues of plants. Wiegand (54, 55) has given an excellent historical summary of the literature dealing with the formation of ice in plant tissues as a setting for his own investigations in this field. The similarity of the effects of drought and freezing on the cell are apparent; both are, from the standpoint of fundamental physiology, desiccation processes. It is probable, therefore, that some of the internal cell conditions important in drought resistance are also important in frost resistance.

The literature covering previous investigations of the causes of death during the freezing process, and of the bases of frost resistance in plants has been comprehensively reviewed by Abbe (1), Blackman (6), Chandler (7), Rosa (48), Harvey (32), and Newton (45). Only a condensed statement of the more pertinent contributions to our knowledge in these fields will be included here. Muller-Thurgau (43) and Molisch (42) believed that the injury involved in the freezing to death of a cell resulted from the withdrawal of water from the protoplasm, which resulted in its structural disorganization. Maximow (40) thought that death resulted only when the water was withdrawn from the plasma membrane. The withdrawal of water from the cell gradually concentrates the electrolytes in solution in the vacuole. Gorke (10) advanced the theory that this concentration of the electrolytes had a salting out effect on the proteins in the cell sap and protoplasm, and that this was the cause of death. Harvey (32) believed that this salting out effect is inadequate to explain the precipitation of the proteins upon the freezing of a plant juice unless the increase in the hydrogen ion concentration which accompanies a lowering of temperature is taken into consideration, as a contributing or modifying factor.

There are several conditions which may operate in the cell in such a way as to prevent injury in the freezing process. A high osmotic value of the cell sap, or a high force of imbibition in the protoplasmic colloids would tend to prevent movement of water from the cell when crystallization occurs in the intercellular spaces. Newton (45) found the latter condition to be an important factor in the frost resistance of winter hardy wheat. Disorganization of the protoplasmic complex due to the concentration of electrolytes accompanying the withdrawal of water from the cell may be prevented if the proteins have been converted to a less readily precipitated form during the hardening process (Schaffnit, 51; Harvey, 32). Accumulation of sugars in the cells exerts a protective action which checks or prevents the precipitation of proteins (Schaffnit,51; Lidforss,36). Maximow (40) has found that solutions of many non-toxic compounds, both organic and inorganic, which have a low eutectic point relative to the freezing point, exert a remarkable protective action on the freezing of plant tissues which are immersed in them.

This paper reports the results of an investigation upon the range of certain physical properties of leaves and leaf saps in fifty species of plants, representing many of the habitats common to central Ohio. Determinations were made of the water content of the leaves, amount of sap which could be expressed from the leaves under a standard treatment and pressure; and of the osmotic value, percent of solid matter, and colloidal content of the expressed leaf saps. One purpose of this general survey was to secure data upon the range of these physical properties in the leaves of native plants, and to discover the correlations, if any, between habitat or growth form and the physical properties of leaves. The native vegetation of Ohio presents many unsolved problems in the physiology of frost resistance, and in the physiology of relative drought resistance. It is hoped that this study may fulfill a second purpose by serving as a foundation for more detailed investigations upon some of these problems.

II.

Review of Literature.

The only physical property dealt with in this paper which has been extensively studied by previous workers is the osmotic value* of the expressed leaf saps. The first critical work on the determination of the osmotic pressure of leaf saps by the cryoscopic method was that of Dixon and Atkins (8, 9) in 1913. They demonstrated the necessity of freezing or otherwise treating the leaf sample before the sap was expressed in order to obtain representative results. Their earlier work and the work of previous investigators had shown that samples of sap expressed from untreated tissues could not be regarded as typical because samples of sap successively extracted from the same tissue differed in osmotic pressure. This differential expression of the sap is almost completely eliminated if the leaf sample first be frozen.

Harris (16-30, inc.) and his collaborators have made a survey of the osmotic pressures of the expressed leaf tissue fluids of plants from a wide range of habitats. Osmotic pressures were

* A solution such as an expressed plant sap has only a potential osmotic pressure. In this paper this is termed the osmotic value. The term osmotic pressure is retained, however, when discussing the results of investigators who have used this term consistently in their writings.

calculated from the depression of the freezing point of saps expressed after freezing the tissue in an ice-salt mixture. These investigations have been important in determining the range of osmotic pressures in leaf tissue fluids. In general the expressed juices of leaves from plants growing in dry or saline habitats are higher in osmotic pressure than the leaf saps from plants growing where the moisture conditions of the soil are more favorable. Important exceptions to this generalization, the cacti for example, show the inadequacy of explanations of the water relations of plants on the basis of osmotic pressures alone. Ligneous plants, in general, have leaf saps with higher osmotic pressures than herbaceous plants. The osmotic pressure of the expressed sap of parasitic forms is generally, but not invariably, higher than that of the host plant. Epiphytes show lower osmotic pressures than terrestrial forms. Correlated studies upon the electrical conductivity of the expressed leaf saps have shown that this is higher in the tissue fluids of herbaceous species than in ligneous species.

Korstian's study (34) of the "sap densities" of plants in the Wasatch mountains of Utah and vicinity is a recent contribution in this field. The results of this investigation confirm in general those of Harris and his co-workers. Saps from herbaceous plants were found to have lower osmotic values than saps from ligneous species. The sap of shade plants showed lower values than that of sun plants of the same species. Young leaves and shoots showed lower sap concentrations than older tissues. Plants from dry or saline habitats showed a higher concentration of solutes in the expressed leaf saps than plants from habitats where the moisture conditions were more favorable. Correlated studies were made of temperature, moisture content of the soil, and evaporation. The opinion of this investigator is that "——— the sap density may be used as an index of site in correlating the great complex of environmental factors with the physiological responses of the plant." The osmotic value of the sap of a species is not constant. It may be influenced by any of the environmental conditions affecting transpiration, absorption, and food manufacture. The osmotic value of the sap of plants was found to be affected more by fluctuations in the moisture conditions of the habitat than by fluctuations in temperature or light.

III.

METHODS.

1. *Collection and Preparation of Leaf Samples.*

The leaf samples were all collected between the hours of one and three in the afternoon, with the exception of those in Series III, which were collected between eleven and twelve in the morning. The leaf samples were placed, on collection, in thick walled test tubes, one and one-quarter inches in diameter, and eight inches long. The capacity of such a tube is about fifty grams of leaves. Two tubes, or approximately one hundred grams of leaves, were collected for each species. Except when composed of very large leaves, as, for example those of the water lily, such a sample consists of several hundred leaves and this number should be sufficient to insure representative results. Most samples of tree leaves were collected from one individual. From herbs, shrubs, and a few of the smaller species of trees it was impossible to secure an adequate sample from one plant. Whenever this was the case, care was taken to collect the leaf material from individuals growing in the same habitat, and, whenever possible, from closely adjacent plants.

Certain almost obvious precautions were taken in the selection of the leaf samples. Only the blade of the leaf, or if the leaf were compound, only the leaflet blades were collected. When the mid-vein was extremely prominent, as in the leaves of burdock (*Arctium minus*), dock (*Rumex patientia*), and water lily (*Castalia odorata*), it was removed; otherwise the entire leaf blade was used. Only apparently sound and healthy leaves, with a uniform green color, were chosen. Collections were made only when the external leaf surfaces were free from moisture. Samples of evergreen leaves were selected only from leaves produced during the current season's growth.

The leaf samples, collected as described, were used as material for all the determinations except that of the total moisture content of the leaves. The filled test tubes were stoppered tightly with rubber stoppers immediately after the collection of the sample and plunged at once into a freezing mixture of ice and salt at a temperature of about $-20°C$. The tubes were arranged in the freezing bath so that the stoppers were emerged, thus avoiding possible contamination by the ice-salt mixture. Double-walled, galvanized iron buckets, in-

sulated with cotton batting, were found serviceable as containers for the freezing mixture. The tubes were left in the ice-salt mixture from fifteen to twenty hours, in practice usually overnight. If necessary the freezing mixture was renewed.

The effect of freezing the leaf tissue is to increase the yield of sap when expressed under pressure. The sap sample is also more representative when this method is used. Dixon and Atkins (8), while working on means of extracting sap for osmotic pressure measurements, developed this method. These investigators froze the samples in liquid air. Gortner and Harris (11), also primarily concerned with osmotic pressure measurements, substituted the ice-salt bath as a more universally available mode of freezing. The method used in this investigation is essentially that developed by them. Previous workers (André, 2; Marie and Gatin, 39) had shown that successive portions of saps expressed under pressure from unfrozen tissues showed progressively increasing concentrations of solutes as measured by the depression of the freezing point. The following explanation of such results was given by Dixon and Atkins. The initial pressure forces out practically pure water through the differentially permeable cell membranes, which are freely permeable to water, but difficultly permeable to most of the solutes present in the cell sap. With increasing pressure some of the cells burst, contributing their entire complement of sap, as well as their protoplasmic contents to the portion of the sap which has already been expressed. The sap released by the bursting of the cells will contain a higher proportion of solutes than that which is forced out through the protoplasmic membranes. Increase in the pressure will cause the bursting of additional cells, therefore each successive sample will contain more solutes, and will consequently have a higher osmotic pressure. If, however, according to these workers, the tissue first be frozen, the membranes become much more freely permeable; solutes as well as water are expressed, and a more representative, as well as a larger sample is obtained. Furthermore, successive portions of sap expressed from the frozen tissues used by these investigators did not show the increasing concentrations of solutes which occurs when unfrozen tissues are used. Gortner, Lawrence and Harris (12) have shown that successive portions of sap expressed from unfrozen tissues may show decreasing concentrations of solutes, or all of the fractions may show approximately the same concentration of solutes. Newton,

Brown and Martin (46) have shown that there are slight variations in the freezing point depression (hence in the osmotic value) of successive portions of sap expressed from frozen wheat leaves. This variation is much less when the sap is expressed from frozen samples of the leaves than when it is expressed from unfrozen samples, and these investigators point out the fact that the difference in the behavior of these two samples is merely one of degree. These extensions of the results of Dixon and Atkins do not invalidate the freezing method as yielding the most representative results when the osmotic value of the expressed sap is to be measured.

There can be little doubt that the increase in permeability of the cells after the freezing treatment is due to the actual destruction of the cytoplasmic membranes and consequent death of the cells. Deciduous leaves and the leaves of evergreens in the summer condition uniformly show a greater yield of sap from frozen than from unfrozen tissues, showing that some or all of the cells in the leaves have been affected by the freezing treatment.

Knudson and Ginsberg (33) reported that the expressed tissue fluids from the leaves of *Iresine Herbstii* Hook. showed about the same value for osmotic pressure when they were prefrozen in the ice-salt mixture as when the preliminary treatment consisted in freezing them in liquid air. It would be dangerous to attempt to generalize this result to other species, however, until more data are available.

Another advantage of the prefreezing treatment is that there is little likelihood of any change taking place in the sample after it is once frozen. When samples must be collected at considerable distances from the laboratory, as was the case in this investigation, it is the only method which may be used with any assurance that considerable physical and chemical changes have not taken place in them before they can be used in experimental work.

Tubes containing frozen leaf samples were thawed by exposing to room temperature for about fifteen minutes. All samples were weighed to determine the exact amount of leaf material used in each determination. The weighing was carried out in the tubes in which the sample was collected. After weighing the tubes were kept in ice water for the short interval of time which elapsed before the sap was expressed.

2. *Expression of the Leaf Tissue Fluids.*

Knudson and Ginsberg (33) have demonstrated experimentally that the osmotic value of plant juices expressed from frozen leaf tissues varies with the pressure used in extraction. The concentration of solutes in a plant sap is dependent upon the pressure used in extracting it, and the physical and chemical properties of that sap will vary accordingly. It is important that the extractions of plant saps be made under known pressures. This standardization will make possible a duplication of a determination at any time or place that measured pressures are available. Newton, Brown, and Martin (46) have also emphasized the importance of standardizing the extraction procedure, particularly with respect to the pressure used. A press similar in design to that originated by Knudson and Ginsberg was first used. This consists of a cylinder and tightly fitting piston with a delivery pipe at the base. For the dimensions and details of the construction of this piece of apparatus, the original paper should be consulted.

The method of using this press will be briefly described. A fifteen inch square of linen cloth was folded twice and placed in the bottom of the cylinder. The sample of leaves was transferred from the tubes in which it was collected and frozen, and placed on the cloth in the bottom of the press cylinder. The cloth was drawn up around the sample and the piston inserted. Pressure was applied to the top of the piston by means of a materials testing machine* and gradually brought up to five thousand pounds to the square inch. It should be noted that the pressure was merely brought up to five thousand pounds and not maintained at that figure after it once had been attained. After the maximum has been attained there is a slow diminution in the pressure to which the leaf sample is subjected, due to the gradual exudation of sap through the delivery pipe. A uniform practice was followed of allowing the press to drain for five minutes after the maximum pressure had been attained. In some of the earlier determinations muslin cloth was used instead of linen, but the tensile strength of the muslin was not always sufficient to withstand the pressures used, consequently it frequently ruptured with an accompany-

* The writer is indebted to Professor Horace Judd, of the Department of Mechanical Engineering of the Ohio State University, for permission to use this machine.

ing extrusion of leaf material through the delivery pipe. This style of press proved heavy and cumbersome for general work. Furthermore, the piston frequently became stuck in the cylinder, and could only be removed by mechanical means, an awkward and tedious procedure. The atmospheric pressure on a piston with this top area (12.56 square inches) is about one hundred and ninety pounds and this is sufficient to prevent its removal by hand whenever the leaf sample becomes tightly enough wadded into the bottom of the press cylinder to prevent the ingress of air through the delivery pipe. For these reasons a smaller and improved press was designed for these investigations. The essential design is similar to that of the press previously employed. The piston and cylinder were turned from steel and ground to fit within .0015 inch. The piston is five and one-half inches high and three inches in diameter. The cylinder is six inches high and has an outside diameter of four inches. Its inside diameter is three inches, and it is bored out to a depth of four and one-half inches.

A groove cut around the inside wall of the cylinder one inch from the top was designed to act as a liquid seal and prevent the upward movement of sap past that point. A groove was also cut around the base of the cylinder, adjacent to the vertical wall. This was designed to collect the sap and feed it to the two delivery pipes on opposite sides of the cylinder. These tubes were made of brass and threaded into place so that they might easily be removed for cleaning. An important addition to the press is a metal disk one-quarter inch thick, of a diameter slightly smaller than the inside diameter of the cylinder. On the lower side of this disk are eight equally spaced radiating grooves, one-eighth inch wide and one-eighth inch deep. Six one-sixteenth inch holes, spaced at equal intervals, are drilled through the metal disk into each groove. The removal of both the piston and the metal disk from the cylinder is facilitated by means of handles which screw into place. A press of these dimensions will readily accommodate a hundred gram leaf sample.

The employment of the metal disk described above makes it possible to practically dispense with the use of cloth for wrapping the samples. Two or three disks of linen cloth, cut to a slightly larger diameter than the cylinder, and placed on top of the metal disk are all that is required. In fact, when leaves containing considerable amounts of ligneous tissue are used, the linen disks can be dispensed with. The leaf sample is placed

directly on top of the cloth disks. The elimination of cloth for wrapping the sample is one of the advantages of the re-designed press. It is also convenient in that it is small, light and easily handled, and that all parts may be readily cleaned. If used with reasonable care, the piston never sticks in the cylinder. This type of press is well adapted for the collection of samples of expressed sap at successively increasing pressures and for determining pressure dehydration curves for plant tissues. It may also be used to determine the pressure necessary to express a given quantity of sap. Presses of this design may be built of any convenient size. They are superior to any type within the writer's experience for general work in the expression of plant tissue fluids.

The expressed plant juices were still cool as they came from the press. They were collected in porcelain evaporating dishes, poured immediately into graduated test tubes, and kept in ice water until used for further determinations. Of course it is impossible to maintain the integrity of an expressed leaf fluid for even a second after it is forced from the tissues. Oxidations occur as soon as the fluid comes in contact with the air, sometimes accompanied by a conspicuous darkening of the sap. Internal changes in such a complex system of physical and chemical equilibria as a plant sap are bound to occur. This is particularly true since many of the compounds brought together in the sap were separate in the leaf tissues. Chemical and physical changes in the saps were mimimized as far as possible by keeping them in stoppered tubes in ice water. Furthermore, determinations of the properties of the saps were made as soon as practicable after their extraction. The time intervening between the expression of the sap and the completion of determinations upon that sap was never more than four hours, and was usually much less. Tests made of the osmotic value of several saps kept under these conditions showed that no appreciable change in this property, at least, occurred within this period of time.

Equal weights of leaves from different species of plants showed marked differences in the yield of sap. The range, for a hundred gram sample was from two cubic centimeters in *Tsuga canadensis* (hemlock) to sixty-seven cubic centimeters in *Bryophyllum calycinum*. The exact amount of juice expressed was measured as accurately as feasible. The delivery pipes were unscrewed and drained. When linen cloth was used for

wrapping the samples before placing them in the press, the amount of water absorbed by the cloth was determined by drying in an oven at 103°C. No appreciable error is incurred by considering the weight of the water evaporated as equal to the amount of sap which had been absorbed by the cloth. Even when the redesigned press was employed and the only cloth used consisted of several linen disks, this correction was made. The amount of sap absorbed by three of these disks, the number usually employed, seldom exceeds one cubic centimeter, however, and for many practical purposes can be disregarded.

The fact that equal sized samples of different species of leaves yielded varying amounts of sap when subjected to the same treatment and pressure made it important to carry out determinations of the total water content of each leaf tissue. With these data available it is possible to determine how much of this variation in the amount of water which can be expressed is due to variations in the water content of the leaf tissues, and how much of it is due to the resistance of those tissues to dehydration under pressure. The total water content of the leaves was determined by drying approximately twenty-five gram samples of leaf tissue to constant weight in an oven at 103°C.

3. *Determination of the Osmotic Value of the Expressed Leaf Saps.*

The determination of the depression of the freezing point of the expressed leaf juices was made by the classical method originated by Beckmann (5). Gortner and Harris (11) have discussed the application of this method to plant saps and have suggested several modifications in apparatus and technique which greatly facilitate the determination. Several of these suggestions were followed in the present work; insulation of the freezing bath, use of a metal air jacket, and employment of auxiliary freezing baths.

A double-walled, galvanized iron can, insulated with cotton batting, makes a convenient freezing bath. This bucket is seven inches high, three inches in internal diameter, and has a space between the walls of one and one-half inches. A metal cover is also provided through which is inserted a stopper holding a metal air jacket. A brass tube, one and one-eighth inches in internal diameter, and about six inches long, stoppered

at the bottom, makes a satisfactory air jacket. This air jacket is plugged with a rubber stopper through which is inserted a hard glass freezing tube. Culture tubes, 18 x 150 mm. are well adapted for this purpose. A Haidenhain thermometer was used with this freezing point apparatus. It is less cumbersome than the more familiar Beckmann type and the smaller size of the bulb permits the use of smaller freezing tubes and smaller samples of sap. As little as four cubic centimeters of sap will suffice with this arrangement. Moreover, the Haidenhain thermometer is less susceptible to change in the zero point than the standard Beckmann type*. This slow change has probably been correctly explained by Atkins (3) as due to the distillation of mercury from the curved surface of the hanging drop at the top of the reservoir of the Beckmann thermometer to the flat surface at the lower end. It is well known that the vapor pressure of a convex surface is greater than that of a flat surface. In the Haidenhain model there is only one mercury surface so that such a distillation with a consequent change in the zero point of the thermometer is impossible. There are slight changes in the zero point of this type, however, due probably to minute fluctuations in the volume of the thermometer bulb, probably due, in part, at least, to aging of the glass. For this reason the freezing point of distilled water was checked every few days throughout the season's determinations. The variation in three months was found to be less than .01 degree. The freezing tube is also provided with a rubber stopper and stirring rod. A stirring rod may also be provided for the freezing bath if desired, but the insulation makes this unnecessary.

In practice, several auxiliary baths were used, depending upon the number of determinations to be made. These baths were water-ice-salt mixtures, cooled to $-1°$ or $-2°$C. Wide-mouthed thermos bottles (internal diameter two inches) make very satisfactory containers for these auxiliary baths. About five cubic centimeters of sap, centrifuged to remove suspended debris, were poured into a freezing tube, a thermometer and a stirring rod inserted through the stopper, and the tube placed in one of the pre-cooling baths. Several samples may be cooling at one time. After the mercury had fallen into the capillary, the freezing tube and attached thermometer were transferred to the freezing bath. This may be at a temperature of -10 to -15

* Improved models of the Beckmann thermometer are now on the market of such construction that change in the zero point is at a minimum.

degrees. The fact that the mercury is already low in the capillary and that the freezing tube is surrounded by a jacket of air permits the use of such a low temperature. The sample was slowly stirred until undercooling and susbequent freezing had taken place. A thermometer reading must be taken both for the point of maximum undercooling (convergence temperature) and for the freezing point. Rapid agitation of the sap sample with the stirring rod was sometimes resorted to to induce crystallization. Usually, however, this was unnecessary. With this technique, after a little practice, from five to six determinations may be made in one hour. Between determinations the metal air jacket was kept plugged with a rubber stopper in order to keep the air at a low temperature. It is also advisable to provide the air jacket with a thermometer so that a check may be kept on its exact temperature.

Observed freezing point depressions were corrected for undercooling by the formula of Harris and Gortner $D = D' - .0125uD'$ (14). Harris (31) has recently expanded it into a table which greatly facilitates corrections for undercooling. The corrected freezing point depression readings were converted into equivalent osmotic values from Harris and Gortner's tables (14, 15). The direct relation between the freezing point and the osmotic pressure is expressed by the equation $O.P = 12.06D—0.021D^2$, (Lewis 35). The table cited is based on this formula.

4. *Determination of the Percent of Solid Matter and the Colloidal Content of the Leaf Saps.*

Gortner and Hoffman (13) were the first to point out that the colloidal content of a plant sap may be an important physical property. A method for determining the relative amount of hydrophilic colloids present in plant saps has been introduced by Newton and Gortner (44). This method expresses the colloidal content in terms of "bound water". The method will be briefly reviewed, but for details the original paper should be consulted.

The solid matter content of the sap is first obtained by the refractometer method of Gortner and Hoffman (13). An Abbe-Hilgard refractometer was used*. The percentage of water in the sap was obtained from published tables of the United States Bureau of Standards (56), and the total solids calculated

* Lent to the writer by courtesy of Dr. Alpheus W. Smith, of the Department of Physics of the Ohio State University.

from these data. This method seems to be at least as accurate as the alternative method of desiccation *in vacuo*, and is more economical of time and apparatus.

The freezing point depression of the sap is determined. A fresh sample of the sap containing exactly ten grams of water is then weighed out. To this is added 3.422 grams of sucrose, just sufficient to make a weight molecular solution. The sucrose crystals are first ground fine in a mortar to insure rapid solution. The freezing point depression of a weight molecular solution of sucrose is 2.085 degrees. This value is in excess of the usual molecular freezing point depression (1.86) because of the hydration of the sucrose molecules and consequent reduction in the amount of available solvent. Six molecules of water are bound to each molecule of sucrose (Scatchard, 50). Hence one mol of sucrose binds six mols or 108 grams of water. The net result is one mol of hydrated sucrose dissolved in 892 grams of water. Since this is more concentrated than a molecular solution the freezing point depression is greater than the molecular lowering. After the addition and solution of the the sucrose, the freezing point depression of the sap sample is redetermined. This value is usually found to be greater than the orignal freezing point depression plus 2.085. This excess depression is considered to be due to the fact that part of the water is "bound" by hydrophilic colloids present in the sap and is thus unavailable as a solvent. That is, the sucrose solution is more concentrated than a weight molecular solution because the 8.92 grams of water not bound by the sucrose are not all free to act as a solvent, and consequently the freezing point depression of the solution is greater.

The percent of bound water is calculated as follows:

$$\text{Bound water} = \frac{D' - (D + 2.085)}{D' - D} \times 89.2.$$

D is the freezing point depression of the expressed juice, D' is the freezing point depression after the addition of the sucrose, 2.085 is the molecular freezing point depression for sucrose, and 89.2 is the percentage of free water in a weight molecular solution of sucrose.

This method is adapted to numerous modifications in technique. In general, it consists in producing a known change in the concentration of a solution for which the depression of the freezing point is known. The resulting change in the depression

of the freezing point is measured and compared with the change calculated for the known change in concentration. If the depression of the freezing point is greater than the theoretical expectation, it measures the amount of water unavailable for solution, i. e. the bound water.

If the bound water be absorbed, as Newton and Gortner's work seems to indicate, this method should give an approximate measure of the surface area of the colloids available for absorption. It is not a measure of the mass of material present in the sap in the hydrophilic colloid state, as the same mass may have different surface areas depending upon the degree of dispersion and shape of the particles.

The possible sources of error in this method have been discussed by its originators. The validity of the method as a measure of the hydrophilic colloid content of a sap rests upon the assumption that there are no other substances present in the sap which bind appreciable amounts of water. The only non-colloidal substance which is likely to be present in plant saps in such amounts as to bind appreciable amounts of water is sucrose. An initial concentration of sucrose in the sap equal to a five percent solution, for example, would bind about 1.6% of the water present. This possible source of error is not emphasized by the originators of this method, but in the interpretation of the results of an investigation covering a variety of plant saps it should be borne in mind.

IV.

The Range of Physical Properties in the Leaves of Native Ohio Plants.

This investigation was conducted during the summer of 1925. Leaf samples were taken from about fifty species of plants, practically all of which are native to central Ohio. Determinations were made of the total water content of the leaves, the amount of sap expressed from the leaves under a standard treatment and pressure, and of the osmotic value, percent of solid matter, and colloidal content of the expressed leaf juices. A standard method of freezing all samples in an ice-salt mixture overnight, and expressing the sap under 5000 pounds of pressure was followed, as described in the preceding section. Unless this pre-freezing and high-pressure extraction method were used, the leaves of many of the ligneous species

investigated failed to yield sufficient volumes of sap to permit these determinations. This method unquestionably yields representative results for measurements of the osmotic value and solid matter content of the expressed tissue fluids. The pre-freezing treatment presumably has a precipitating effect on the cell proteins, and this source of error may affect the determination of the colloidal content of the sap.

A summary of the data resulting from these determinations is presented in Table 1. Series I represents plants from a mixed mesophytic ravine forest and an adjacent oak hickory forest near Columbus. Series II consists of plants from near Sugar Grove, Ohio. Leaves of *Rhododendron maximum* (rhododendron) and *Tsuga canadensis* (hemlock) were collected from the north facing slope of a deep, moist ravine. This habitat is abundantly supplied with soil water. All the other plants in this series grew on the top of a high, well-drained ridge. This was the driest habitat studied. This ridge is forested with an open forest of mixed *Pinus rigida* (pitch pine) and *Pinus virginiana* (scrub pine), which is evidently the pioneer tree association in a secondary succession. The shrubs and younger trees are all deciduous with the exception of the broad leafed evergreen, *Kalmia latifolia* (mountain laurel). Series III was taken from the banks of the Big Walnut Creek near Columbus. Series IV represents bog plants and hydrophytes from Buckeye Lake and the associated bog. *Alnus rugosa* (smooth alder), *Pyrus arbutifolia* (chokeberry), *Vaccinium macrocarpon* (cranberry), *Gaylussacia baccata* (black huckleberry), and *Osmunda cinnamomea* (cinnamon fern) were taken from the bog proper. This bog is surrounded by the artificially formed Buckeye Lake and the remaining plants in the series, with the exception of *Castalia odorata* (water lily), grow on the margin between the bog and lake. They are typically pond or swamp margin plants. *Castalia* was taken as an example of a submerged hydrophyte with floating leaves. Series V consists of some common herbaceous weeds. Series VI was taken from the garden and Series VII from the greenhouse of the Department of Botany.

The date of collection is given for each sample. The columns of data, in order, represent the percentage of water in the leaves, the number of cubic centimeters of sap expressed per hundred grams of sample under a pressure of 5000 pounds per square inch, the percent of the total water present which is expressed

as sap under this pressure, the osmotic value of the expressed sap in atmospheres, the percentage of total solids in the expressed sap, and the percentage of bound water in the expressed sap. Blanks appear in the table only where the volume of sap obtained proved inadequate for the missing determinations, or where accidents occurred to the samples.

Discussion will be restricted largely to the range of these physical properties in the species, and over the variety of habitats studied. The water content of the leaves ranges from 51.9% in *Fagus grandifolia* (beech) to 92.7% in *Bryophyllum calycinum*. The averages for the plants studied are trees 60.1%, shrubs 63.7%, and herbs (exclusive of the two species taken from the greenhouse) 76.4%.

The number of cubic centimeters of sap which could be expressed from one hundred grams of leaf tissue under a pressure of 5000 pounds to the square inch varied from two in *Tsuga canadensis* (hemlock) to sixty-eight in *Bryophyllum calycinum*.

That the volume of water expressed is based, in part, at least, on other factors than the water content of the leaves is shown by the fact that the percent of the total water which can be expressed as sap shows a remarkable variation among the different species studied. The range is from 3.4% in *Tsuga* to 88.0% in *Helianthus*. The volume of water which can be expressed from a sample of leaves by pressure may have an important physiological significance. This volume will depend upon the water content of the leaf tissues, the mechanical resistance offered by the tissues to pressure, and the affinity of the tissues for water. In studies of seasonal variations in the amounts of sap which can be expressed from mature leaves, variations in the first two of these factors are frequently so small as to be negligible. Newton (45) found that it was impossible to express appreciable amounts of sap from prefrozen samples of winter hardy wheat during the winter months. During the summer, however, relatively large amounts could be expressed. The writer has found a similar seasonal variation in the amounts of sap which can be expressed from the leaves of native Ohio evergreens. The data on which this statement is based will be published in a subsequent paper. It seems clear, as Newton has pointed out, that this phenomenon must be due to seasonal shifts in the amount and physical condition of the intracellular colloids. It is his belief that larger amounts of water are bound by these colloids in the winter than in the

summer, due probably to the fact that most of the cell colloids are in the gel condition during the winter months. This is probably an important factor in the frost resistance of species with evergreen leaves. Young leaves, in which vacuolation of the cells is not yet complete, should offer a greater resistance to the expression of sap under pressure than mature leaves, since larger proportions of the water in the cells of young leaves are bound by molecular forces. This may account for the fact that the young leaves of some species are more resistant to freezing than mature leaves. No critical experimentation has yet been carried out upon the relative resistance offered by young and mature leaves to the expression of sap under pressure.

The data on osmotic value corroborate the results of earlier investigators in regard to the relation between this property and growth form. Trees in general have higher osmotic values for the expressed leaf sap than herbs, and shrubs occupy an intermediate position. The three groups are by no means mutually exclusive, and for some herbs the osmotic value of the expressed sap was found to be almost as high as the most extreme found in trees. The average for herbs, exclusive of those taken from the greenhouse, is 13.6 atmospheres, for shrubs 16.0 atmospheres, and for trees 17.2 atmospheres. The range for ligneous species is from 11.79 atmospheres in *Rhododendron maximum* (rhododendron) to 21.30 atmospheres in *Populus deltoides* (cottonwood). There is no marked correlation of the osmotic value of the expressed leaf sap with habitat, and Korstian's conception of such a relation is not substantiated. Of course it should be recognized that the extremes of habitat represented in the Ohio flora do not approach the extremes found in Utah, where Korstian's work was done. On the other hand there is clearly a marked difference in terms of environmental factors of a habitat on the margin of a stream, represented by Series III, and one at the summit of a well drained and usually dry ridge, represented by most of the plants in Series II. Yet the two habitats are scarcely distinguishable on the basis of the osmotic values of the expressed leaf saps. The determinations for the trees of the dry ridge average slightly more than one atmosphere higher than the determinations for the trees along the stream margin. Unless differences in the habitat factors are more extreme than the differences between the habitats in central Ohio the osmotic value of the expressed leaf sap is apparently not a safe criterion of site.

The percent of total solids in the expressed saps shows a range of from 1.3% in *Zebrina pendula* (wandering jew) to 20.15% in *Rhus copallina* (dwarf sumac). The average for the plants studied in this investigation are 8.0% for herbs, 12.8% for shrubs, and 13.0% for trees. It appears that trees and shrubs, in general have a higher percent of solid matter in the expressed leaf sap than herbaceous plants.

The percentage of bound water in the expressed leaf tissue fluids ranges from none in *Castalia odorata* (water lily), *Osmunda cinnamomea* (cinnamon fern), and *Zea mais* (corn) to 23.4% in *Pinus rigida* (pitch pine). The average for the plants studied in this investigation are 12.6% for trees, 8.7% for shrubs, and 5.7% for herbs. The average for trees is distinctly higher than that for shrubs, and the average for shrubs is, in turn, markedly higher than that for herbs. The range of variability of this physical property of the expressed saps within each of these three groups is greater than for the other two physical properties of the sap studied. Apparently there is no correlation between the percent of bound water in the sap and the habitat.

It is clear, that over the range of habitats studied, the physical properties of the leaves are more closely correlated with growth form than with the factors of the environment. Herbaceous plants, in general, have a higher leaf water content than ligneous plants. The sap expressed from the leaves of herbaceous plants usually shows a lower osmotic value, a lower percentage of solid matter, and a smaller percent of bound water than the sap expressed from the leaves of ligneous plants. Shrubs usually occupy a position intermediate between trees and shrubs in regard to these measurements.

TABLE I.

SUMMARY OF THE PHYSICAL PROPERTIES OF LEAVES AND LEAF SAPS.

	Date	Plant	Water Content of Leaves, Percent	Sap Yield cc. per 100 Grams	Percent of Total Water Expressed	Osmotic Value of Sap in Atmospheres	Total Solids Percent	"Bound Water" Percent
		SERIES I.						
1	Aug. 3	Quercus alba	56.1	24	42.7	20.39	14.3	0.9
2	Aug. 6	Quercus imbricaria	55.7	6	10.7			
3	Aug. 12	Celastrus scandens	76.4	59	77.2	16.26	10.7	11.4
4	Aug. 12	Cercis canadensis	71.1	40	56.2	12.52	7.5	11.6
5	Aug. 12	Benzoin aestivale	77.7	54	69.4	13.06	5.7	6.3
6	Aug. 12	Quercus velutina	56.9	7	12.3			
7	Aug. 30	Fagus grandifolia	51.9	18	34.6	18.17	11.2	
8	Sept. 18	Acer rubrum	55.8	27	48.3	17.27	15.5	7.4
9	Sept. 14	Acer saccharum var. nigrum	58.2	28	48.1			
10	Sept. 14	Sassafras variifolium	61.4	23	37.4	20.42	16.8	10.8
11	Sept. 14	Carya glabra	57.3	18	31.4	19.74		
		SERIES II.						
12	Aug. 5	Rhododendron maximum	62.0	44	71.0	11.79	8.8	5.1
13	Sept. 15	Tsuga canadensis	57.9	2	3.4			
14	Aug. 8	Kalmia latifolia	55.7	21	37.7	18.14	13.1	8.6
15	Aug. 8	Acer rubrum	58.4	25	42.8	15.72	12.9	5.8
16	Aug. 8	Oxydendrum arboreum	75.2	53	70.4	18.69	15.0	18.4
17	Aug. 23	Gaultheria procumbens	62.0	32	61.5	15.06	11.5	7.5
18	Aug. 23	Smilax glauca	82.7	44	53.2	18.40	12.0	6.4
19	Aug. 23	Rhus copallina	63.0	45	71.4	19.02	20.1	4.3
20	Aug. 23	Cornus florida	64.8	45	69.4	17.34	11.9	8.7
21	Sept. 15	Pinus rigida	66.2	45	67.9	18.21	14.8	23.4
22	Sept. 18	Acer rubrum	56.5	30	53.0	15.04	14.9	6.1
23	Sept. 24	Sassafras variifolium	70.9	25	31.7	15.22	11.1	
24	Sept. 24	Castanea dentata	59.9	16	26.7			

		SERIES III.						
25	Aug. 20	Acer saccharinum	58.3	27	46.3	14.54	11.9	5.0
26	Aug. 20	Salix alba	63.3	36	56.8	14.22	11.4	10.2
27	Aug. 20	Platanus occidentalis	.....	45	.....	12.08	8.2	5.2
28	Sept. 14	Populus deltoides	65.0	43	66.1	21.30	16.6	10.3
		SERIES IV.						
29	Aug. 17	Alnus rugosa	55.1	29.	52.6	17.51	13.6	6.9
30	Aug. 17	Pyrus arbutifolia	59.2	6	10.1	.....	.....	.....
31	Aug. 26	Vaccinium macrocarpon	62.4	40	76.3	19.22	17.5	12.8
32	Aug. 26	Gaylussacia baccata	55.7	33	59.2	19.08	17.4	9.8
33	Aug. 26	Osmunda cinnamomea	74.9	55	73.4	14.88	8.4	0.0
34	Aug. 26	Hibiscus Moscheutos	68.7	48	69.8	12.98	9.8	10.6
35	Sept. 20	Decodon verticillatus	78.1	40	51.2	14.18	7.8	9.8
36	Sept. 20	Sagittaria latifolia	81.5	30	36.8	13.24	7.7	7.3
37	Sept. 20	Typha latifolia	77.9	24	30.8	.....	.....	.....
38	Sept. 20	Polygonum amphibian	71.9	54	75.1	11.80	7.1	5.3
39	Aug. 26	Castalia odorata	75.9	64	74.5	14.74	8.4	0.0
		SERIES V.						
40	July 16	Taraxacum officinale	87.8	.....	.....	13.58	5.2	5.1
41	July 19	Abutilon Theophrasti	68.2	.....	.....	13.04	9.4	3.6
42	July 23	Saponaria offinalis	78.8	.....	.....	13.35	7.6	11.8
43	July 23	Arctinum minus	87.0	.....	.....	10.84	.....	11.2
44	July 24	Rumex Patientia	91.6	.....	.....	6.68	3.4	6.9
45	Sept. 14	Verbascum Thapsus	71.3	51	71.0	13.42	12.5	1.8
46	Sept. 18	Poa pratensis	77.6	64	82.4	13.60	5.1	3.8
		SERIES VI.						
47	Aug. 30	Helianthus annuus	62.5	55	88.0	18.82	8.1	7.8
48	Aug. 30	Zea mais	68.2	48	70.3	19.31	11.1	0.0
		SERIES VII.						
49	July 20	Zebrina pendula	89.2	.....	.....	4.81	1.3	5.0
50	Nov. 9	Bryophyllum calycinum	92.7	68	72.8	5.79	4.5	2.3

V.

SUMMARY.

1. A study has been made of the range of certain physical properties of the leaves and of the expressed leaf saps from plants native to central Ohio. Determinations have been made of the water content of the leaves, the yield of sap from the leaves under standard treatment and pressure; and of the osmotic value, solid matter content, and colloidal content of the expressed leaf saps.

2. A specially constructed press of the cylinder and piston type is described which involves certain new principals in design. This press has given satisfactory results for the expression of leaf tissue fluids from a variety of plants.

3. The numerical range of the physical properties of leaves and expressed leaf saps has been adequately summarized in Table I. The physical properties of the leaves of any species may be regarded as taxonomic characters, variable, of course, within certain limits depending upon the environment of the plants, and the maturity of the leaves.

4. No general correlation between the physical properties of leaves and habitat could be discovered. The osmotic value of the expressed leaf sap cannot be taken as a safe criterion of site over the range of habitats found in central Ohio.

5. The physical properties of the leaves of herbaceous plants as a class are quite different from those of ligneous plants as a class. The leaves of herbs usually have a higher water content than the leaves of ligneous plants. Sap expressed from the leaves of herbs usually has a lower osmotic value, lower solid matter content, and a lower colloid content (measured as bound water) than sap expressed from the leaves of trees and shrubs. Shrubs, in general, show values for these physical properties intermediate between those for trees and herbs.

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VI.

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